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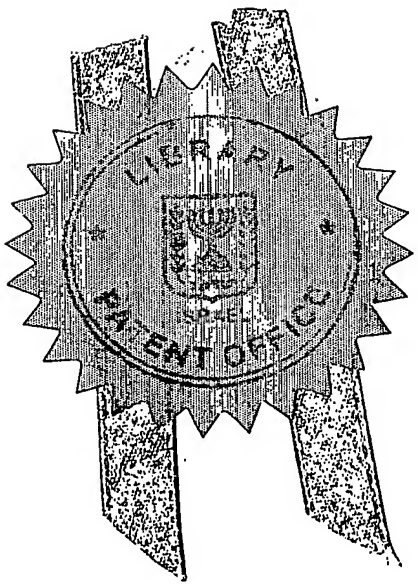

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of an invention the title of which is: — ששמה הוא:

(בעברית) (Hebrew): תרכובת להשתלה בעצם

(באנגלית) (English): BONE GRAFT COMPOSITE

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Signature of Applicant

היום 26 בחודש דצמבר שנת 2002
This 26 of December of the year 2002

For the Applicants,

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Patent Attorney

Ref.: PRO/020/ IL

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תרכובת להשתלה בעצם

BONE GRAFT COMPOSITE

PRO/020/IL

Bone Graft Composite

Field of the Invention

The present invention relates to the field of tissue engineering and more specifically to a composite comprising a synthetic apatite and at least one anti-resorptive agent introduced into the composite *ab initio,* said composite being suitable for use as a bone implant or cement, a method of preparing the composite and uses thereof.

Background of the Invention

Tissue engineering may be defined as the art of reconstructing mammalian tissues, both structurally and functionally (Hunziker, Osteoart. Cart., 10:432-465, 2002). In vitro tissue engineering generally includes the delivery of a polymeric or ceramic scaffold that serves as an architectural support onto which cells may attach, proliferate, and synthesize new tissue to replace tissue loss due to disease, trauma or age. Alternatively, an orthopedic or periodontal prosthesis is implanted to replace lost tissue. Innovations in orthopedic surgery include a vast array of materials that are biocompatible and provide mechanical stability, controlled release of bioactive agents and a scaffold for cell anchorage.

Bone is a unique type of tissue made up of an inorganic mineral phase and cellular and extracellular matrix phases. Bone is a vital organ that undergoes modeling and remodeling wherein old bone is lost (resorption) and new bone is added (formation/replacement).

In children and young adults, bone modeling results in the growth and increase in density of the skeleton. In adults, remodeling normally results in no net change in skeletal size since bone replacement matches bone resorption. Osteoporosis and related diseases ensue when bone resorption exceeds bone replacement.

When damaged or lost by disease or trauma, bone has an inherent capacity for repair and regeneration but the renewed bone is often fragile and not weight-bearing. Bone restoration or replacement is a viable consideration in indications including osteopenia, osteoporosis, bone tumors, spinal fusion and fractures.

Restoring or enhancing fractured, damaged, or degenerated skeletal bone with prosthetic implants made of biocompatible materials is well known in the art. Many materials have been suggested for bone graft, specifically synthetic materials that avoid the harvesting problems associated with autologous material and the health risks associated with allogenic material. Inorganic material such as calcium phosphate (CaP) has been utilized as bone and dental

fillers (reviewed in LeGeros, Clin Orthop 395:81-98, 2002). Apatite, a particulate calcium phosphate, is particularly appealing by virtue of the fact that it is the naturally occurring mineral component in bone and teeth. Bone apatite exhibits low crystallinity due to the presence of magnesium and carbonate (CO_3) ions. Lack of crystallinity in apatites is associated with increased solubility in vivo. Hydroxyapatite, in contrast, exhibits high crystallinity and represents a small component of natural bone. Bone graft materials comprising CaP or hydroxyapatite have been disclosed for use as bone grafts and implants.

Additionally, anti-resorptive agents such as bisphosphonates have been widely used to prevent bone resorption and lower fracture risk in osteoporosis and other diseases exhibiting osteolytic processes.

Use of an implant in combination with an anti-resorptive for the release of bisphosphonate or for preservation of implants has been disclosed.

Denissen et al (Bone Miner. 25:123-134, 1994) describe the use of bisphosphonate impregnated ceramic hydroxyapatite implants for the maintenance of bone mass following tooth extraction due to oral disease.

US Patent 5,733,564 teaches a method of treating endo-osteal materials with a bisphosphonate solution to enhance biocompatibility of prostheses. Endo-osteal prostheses, with or without ceramic coatings, are immersed in a bisphosphonate solution.

PCT patent application WO 00/47214 discloses anti-resorptive bone cements, comprising one or more anti-resorptive agents, preferably a bisphosphonate. The anti-resorptive bone cements are useful for filling bone voids and bonding prosthetic devices to bone.

PCT patent application 02/062352 discloses a method for increasing bone density in an individual comprising a drug delivery device and a bone density-modulating drug.

There remains a need for material having superior biological and physical properties for use as a bone graft in orthopedic indications. In particular, there remains a need for a synthetic biocompatible material exhibiting delayed resorption and the ability to preserve adjacent host bone tissue. Existing bone graft implants or bone substitute materials are prepared by admixing various preformed apatites (calcium phosphate materials) with an anti-resorptive or by impregnating them with an anti-resorptive agent. The art has not heretofore provided a synthetic apatite and anti-resorptive agent composite material wherein the anti-resorptive agent is included *ab initio*.

Summary of the Invention

It is an object of the present invention to provide a biocompatible bone graft composite material comprising a synthetic apatite and at least one anti-resorptive agent. It is another object of the present invention to provide a composite having advantageous biological and physical properties useful in orthopedic, periodontal and craniofacial applications. It is yet another object of the present invention to provide a bone graft composite that exhibits delayed resorption. It is yet another object of the present invention to provide a bone graft composite that delays the rate of resorption of adjacent bone. It is a further object of the present invention to provide a bone graft composite that is useful as bone cement. It is a further object of the present invention to provide a pharmaceutical composition comprising the bone graft composite. It is a further object of the present invention to provide a method of using the bone graft composite. It is yet a further object of the present invention to provide a kit comprising the bone graft composite.

These and other objects will be apparent from the description, figures and claims that follow.

Although numerous compositions comprising calcium phosphate minerals are known in the art, none has proven entirely satisfactory in meeting the criteria required for successful tissue engineering. The inventors of the present invention have found, quite surprisingly, that co-crystallizing a liquid mixture of calcium and phosphate ions, an amino acid, a carbonate and at least one anti-resorptive agent *ab initio* produces a novel composite material. The composite of the present invention provides a superior space filling material for use in orthopedic, periodontal and craniofacial applications where bone preservation is required. The composite of the present invention also provides a bone graft material that exhibits retarded or delayed resorption. The composite of the present invention further provides an anti-resorptive cement for bonding prosthetic devices to bone in skeletal and dental reconstructive surgery.

The composite may be used as a powder, formulated as a semi-fluid composition or cast into a solid device for use at sites of defective or diseased bone or prosthesis implant sites.

According to one currently preferred embodiment of the present invention, a bone graft composite is provided comprising a synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate and at least one anti-resorptive agent.

According to one currently more preferred embodiment of the present invention the synthetic apatite is a poorly crystalline apatite (PCA). PCA is regarded as a superior bone replacement

material to other apatite materials such as synthetic or natural hydroxyapatite or β-tricalcium phosphate due to the presence of carbonate ions and its similarity to natural bone.

According to another currently preferred embodiment of the invention the at least one anti-resorptive agent is a bisphosphonate or a pharmaceutically acceptable salt or ester thereof.

According to another currently preferred embodiment of the invention the anti-resorptive agent is introduced *ab initio* of the preparation step of the synthetic apatite. Without wishing to be bound by any theory, the presence of the anti-resorptive agent during the formation of the synthetic apatite generates a unique composite material wherein the anti-resorptive agent is intercalated, dispersed or distributed within the crystal structure.

It is now disclosed that resorption of the bone graft and resorption of adjacent bone can be inhibited by the presence of at least one anti-resorptive agent introduced during the process of synthesizing the bone graft material. The composite has attributes that make it particularly advantageous for supporting and preserving bone *in vivo*.

Among the advantageous properties of the composite of the invention:

The composite has superior physical properties, controlled by varying anti-resorptive agents used in the preparation. Desirable properties include crystallinity that resembles that of natural bone, retarded resorption, good mechanical stability, structural integrity and convenient use formulation.

The composite has superior biological properties, controlled by varying anti-resorptive agents used in the preparation. Desirable properties include biocompatibility and inhibition of bone resorption in adjacent bone.

The anti-resorptive agents are selected to impart advantageous attributes to the composite. Without wishing to be bound by theory, the anti-resorptive agents impart a stabilizing property to the composite that may be optimized for each of the diverse applications.

This and other features result in a composite exhibiting advantageous properties including biocompatibility, durability, malleability and ease of administration.

According to one preferred embodiment of the present invention a pharmaceutical composition comprising the bone graft composite is provided. The composition may be formulated into a fluid or semi-solid pharmaceutical composition. According to one more preferred embodiment of the present invention the composition is paste-like. In preferred embodiments the composition is an injectable paste. Viscosity of the injectable paste is

preferably in the range of 10-500 poises, more preferably in the range of 30-200 poises, depending on the application and mode of administration.

According to one currently preferred embodiment of the present invention a pharmaceutical composition is provided comprising a synthetic apatite and anti-resorptive agent composite and at least one carrier having sufficient fluidity to enable injection of the composition to the site of treatment.

According to another currently preferred embodiment of the present invention, the composition of the present invention may be used alone or as a carrier to deliver bioactive agents to the site of the bone defect or lesion.

Accordingly, one currently more preferred embodiment the present invention provides a composite comprising a synthetic apatite, an amino acid in monomeric or polymeric form, a carbonate, at least one anti-resorptive agent and at least one bioactive agent selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents or hormones.

The bone graft composite may be administered as a powder or as a fluid or semi-fluid composition for certain bone disease and injury applications. According to another preferred embodiment of the present invention the pharmaceutical composition is fluid at temperatures below physiological conditions and non-fluid at physiological temperatures. Preferably the composition gels, cures or hardens at between 35°-42°C. This particular property of the composition may be achieved by the addition of certain polymers or other additives to the composite of the invention. Preferably the composition comprises an additive that promotes *in situ* hardening of said composition over about 10 minutes to 24 hours following injection. A non-limiting example of such materials includes collagens such as a gel forming soluble collagen disclosed in WO 00/47130, and non-polymeric compounds such as a non-polymeric esters or mixed esters of one or more carboxylic acids disclosed in US Patent 6,413,536. Another non-limiting example includes the addition of calcium sulfate or calcium phosphate compounds. The composition may comprise about 5-50% w:w calcium sulfate.

According to one currently preferred embodiments of the present invention, the synthetic apatite-anti-resorptive agent composite is useful for anchoring a prosthetic bone or implant to living bone tissue. Preferably the composite is a paste-like material, capable of hardening or setting *in situ*.

Alternatively, a solid bone graft device is preferred in the reconstruction of structural tissues like cartilage and bone, certain applications. A composition comprising the composite of the invention may be molded into three dimensional configuration articles of varying thickness and shape. Accordingly, the present invention provides an implant comprising the composite of the invention further comprising t least one hardener, said implant having a specific shape including a sphere, screw, cube, rod, tube or plate.

In preferred embodiments of the present invention a pharmaceutical composition is provided comprising a composite material comprising synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate, at least on anti-resorptive agent, optionally comprising at least one bioactive agent, further comprising a pharmaceutically acceptable carrier or diluent, optionally further comprising at least one hardener.

The pharmaceutical composition of the present invention is useful for treating orthopedic, periodontal and craniofacial indications wherein there is need to fill a void in a bone, to secure a prosthetic implant to a bone or a need to delivery bioactive agents to the bone or tissue in contact with the bone. Tissue closely associated with bone includes ligaments, tendons cartilage and muscle. The composition is useful for the prevention of bone loss associated with the absence of weight bearing in a bone.

In accordance with the invention use of the composite of the invention for the manufacture of a medicament is provided as a graft for diseased or traumatized bone useful in orthopedic, periodontal and craniofacial indications wherein the composite is provided alone or comprising bioactive agents.

According to one aspect of the present invention the synthetic apatite of the composite is a poorly crystalline apatite. According to one exemplary embodiment the composite is prepared by microwaving calcium and phosphate ions with at least one anti-resorptive agent using a procedure disclosed in US Patent 6,231,607. The powder resulting from that process consists of poorly crystalline apatite (PCA) anti-resorptive agent aggregates having a size of approximately 0.45 μm to 6μm in diameter, more preferably 1 μm-4 μm in diameter. The aggregates are comprised of crystals of approximately 0.20 μm-0.30 μm in size.

Another embodiment of the present invention provides a process for the preparation of a fluid bone composition comprising the poorly crystalline apatite according to US Patent 6,231,067 comprising adding at least one additional anti-resorptive agent, and optionally

adding at least one bioactive agent. The process for preparing the composition comprises sterilizing the powder, adding a sufficient amount of liquid to hydrate and disperse the powder, adsorbing a bioactive agent and preparing the wetted powder for administration. Following the wetting procedure the composition may be optionally filtered to remove excess liquid, thus leaving a paste like material on the filter.

The process comprises the following steps:

a) preparing a liquid mixture comprising ionic calcium, phosphate, at least one amino acid in either monomeric or polymeric form, carbonate, comprising at least one anti-resorptive agent;

b) subjecting said mixture to microwave irradiation;

c) quenching said irradiated mixture;

d) filtering said irradiated mixture so as to separate between the filtrate and a cake;

e) drying said cake;

f) grinding said dried cake into a powder;

g) sterilizing said powder;

h) optionally wetting said sterilized powder with a solution optionally comprising at least one bioactive agent;

i) preparing said wetted powder for administration.

According to one currently preferred embodiment of the present invention the powder resulting from step (f) consists of poorly crystalline apatite (PCA) calcium phosphate-anti-resorptive agent composite aggregates having a size of approximately 0.45 μm to 6μm in diameter, more preferably 1 μm-4 μm in diameter. The aggregates are comprised of crystals of approximately 0.20 μm-0.30 μm in size.

According to one currently preferred embodiment of the present invention the PCA powder composite resulting from step (f) is sterilized in a manner that substantially retains the X-ray diffraction pattern of the powder, preferably by ionization techniques, more preferably by γ-irradiation. The present inventors have found that following sterilization by γ-irradiation the bone graft composite retains its molecular crystal structure, as determined by X-ray diffraction analysis. Preferably the composite retains an X-ray diffraction pattern having an undifferentiated peak of 2 theta=31°-33°.

The bone graft composite further exhibits a calcium to phosphate ratio (Ca/P) similar to that of natural bone. Accordingly, the synthetic apatite may contain cation or anion substitutions. According to one currently preferred embodiment, magnesium ions (Mg^{++}) and/or zinc (Zn^{++}) are added to partly replace the calcium ions, preferably Mg^{++}.

According to a preferred embodiment, a sufficient amount of liquid is added to permit wetting and dispersion of the powder to form a hydrated precursor mixture having a consistency compatible with application to a filtration device. The wetted powder is filtered through a sterile filtration device having pore size enabling retention of the crystalline aggregates on the filter. Preferably, the pore size of the filtration device permits full retention of the bone graft material.

The filtered material retains sufficient fluidity to enable handling without fragmentation or separation of the liquid from the solid phase. Preferably the wetted powder has a consistency or putty or paste. Viscosity of the injectable paste is preferably in the range of 10-500 poises, more preferably in the range of 30-200 poises, depending on the application.

In another embodiment of the present invention, the wetted powder is blended under sterile conditions to a consistency compatible with administration to a site of a defect or lesion or to anchor a prosthetic implant to bone, *in situ*. The paste may be administered manually or with a spreading instrument such as a spatula. More preferably, the wetted powder is inserted into a syringe and is prepared for local administration or injection into the defect site or a prosthesis anchorage site.

In one currently preferred embodiment of the present invention the powder is mixed with a bioactive agent, preferably an antibiotic or anti-inflammatory agent, the powder and the liquid being mixed at a w/w or w/v ratio of about 1:1 to yield a paste-like pharmaceutical composition. In one particular exemplary embodiment 3 gm powder is mixed with 3 ml sterile aqueous solution to yield approximately 5ml paste-like composition.

In another particular exemplary embodiment 3 gm PCA powder are mixed with 20 ml sterile aqueous solution and filtered through a 0.45 μm filter to remove excess liquid to yield approximately 5 ml paste-like material.

In yet another embodiment the paste-like material is prepared for administration. According to one currently preferred embodiment the paste-like material is administered directly to a bone defect. According to one currently more preferred embodiment the paste-

like material is inserted into a syringe for local administration. According to one currently most preferred embodiment the paste-like material hardens in situ within about 24 hours following injection. Alternatively the paste-like material hardens *in vitro* to form a molded implant. Furthermore, the composite may be used as a coating on synthetic or other implants such as pins and plates, for example, in hip replacement procedures. Thus, the present invention further provides implants or medical devices coated with the matrix of the invention.

Representative uses of the compounds and combinations of the present invention include: filling in of bone defects and deficiencies such as those occurring in closed, open and non-union fractures; prophylactic use in closed and open fracture reduction; bone replacement in plastic and dental surgery; anchorage of prosthetic joints and dental implants; elevation of peak bone mass in pre-menopausal women; treatment of periodontal disease and defects, and other tooth repair processes and treatment of other skeletal disorders, such as age-related osteoporosis, post-menopausal osteoporosis, PVA, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis, or any condition that benefits from delayed bone and bone implant resorption. The compounds and combinations of the present invention can also be useful in repair of congenital, trauma-induced or surgical resection of bone (for instance, for cancer treatment).

According to yet another currently preferred embodiment of the present invention provides a kit comprising the disclosed bone graft composition, where the dry and liquid components may be present in separate containers in the kit, or some of the components may be combined into one container.

Brief Description of the Figures

Figure 1 shows the X-Ray diffraction patterns of the bone substitution composites of the invention.

Detailed Description of the Invention

The present invention is directed to a biocompatible composite useful as an implant for treating bone disorders and diseases or as cement to anchor prostheses in certain orthopedic and dental indications.

In principle, an ideal bone implant/cement will exhibit the following properties:

Biocompatible: minimal toxicity and maximal resemblance to natural bone.

Durable: delayed resorption, mechanically strong.

Practical: convenient for use by the medical practitioner.

The present invention provides a composition exhibiting the aforementioned advantageous properties.

Definitions

For convenience and clarity certain terms employed in the specification, examples and claims are described herein.

The term "anti-resorptive agent" as used herein refers to a compound, or drug having the ability to prevent, delay or reduce resorption of an implant or resorption of adjacent bone. Examples of anti-resorptive agents include phosphonates, preferably bisphosphonates and derivatives thereof. Other anti-resorptive agents include estrogens, prostaglandins and calcitonin.

The terms "bisphosphonate" or "bisphosphonic acid" as used herein, relate to those phosphonates or phosphonic acids that have two phosphonate groups attached to the same carbon atom. Based upon their chemical composition bisphosphonates can be classified into nitrogen-containing, N-bisphosphonates, including alendronate, zolendronate and pamidronate and non-nitrogen containing bisphosphonates including clodronate and etidronate. Bisphosphonates are potent inhibitors of bone resorption and are effective in treating diseases and disorders of bone resorption. Many bisphosphonates are known in the art, exhibiting different anti-resorptive potency that can be exploited in the preparation of the composite. For example, risedronate and zolendronate (contain a nitrogen atom in heterocyclic side group) are more potent than etidronate in inhibiting bone erosion. It is to be understood that the bisphosphonates useful herein include as non-limiting examples, e.g., alendronate, clodronate (clodrinate), etidronate, pamidronate, medronate, nedrinate,

tiludronate, zolendronate or combinations thereof. Other non-limiting examples of bisphosphonates have been disclosed in US patents 5,856,314 and 5,338,731.

Furthermore certain bisphosphonates compounds have anti-tumor activity per se (reviewed in Cancer Treat Rev 28(6):305-19, 2002). This feature renders them useful for the treatment of bone metastases in certain cancers. A preferred embodiment of the present invention provides a

The term "biocompatible" as used herein refers to materials having low toxicity, high affinity with living tissues and no unacceptable foreign body reactions in the living body.

A "composite" as used herein refers to a material that is made up of two or more distinct elements. The composite of the invention is unique in that it comprises a mineral phase and an organic phase that are intercalated or interdispersed *ab initio*.

"Apatite" as used herein refers to a crystalline calcium phosphate mineral. "Synthetic apatite" refers to a manufactured crystalline calcium phosphate material. It is to be understood that that a part of the calcium (Ca^{++}) ions may be replaced with other divalent ions selected from the group consisting of Magnesium (Mg^{++}) and Zinc (Zn^{++}). The incorporation of additional or different divalent ions imparts on the composition certain properties which may be advantageous in certain applications.

A composite or composition is considered to be "biodegradable" if it is soluble and erodes *in vivo*. Preferably the composite of the invention remains essentially intact throughout the life of the patient. A non-biodegradable composite is advantageous for maintaining bone integrity in certain diseases and disorders associated with bone remodeling.

The term "resorption" or "bone resorption" refers to the normal process of bone erosion by a group of cells known as osteoclasts. In remodeling, the bone building cells, osteoblasts, infiltrate and fill the resorption sites to form functional bone. Normally, bone resorption equals bone formation. However, in certain diseases and disorders bone resorption surpasses bone formation and bone loss occurs, increasing an individuals risk for osteoporosis and related bone-weakening diseases.

Osteoporosis may manifest itself in patients who are undergoing prolonged treatment with certain drugs such as adrenal corticosteroids, who lead sedentary lifestyles, who suffer from chronic renal disease, and who are malnourished.

The term "osteolysis" refers to the dissolution of bone.

"Diseases and disorders associated with bone remodeling" includes diseases and disorders that are inherited, spontaneous, chronic, metabolic, drug induced or a manifestation of another disease. These include, but are not limited to, osteoporosis, osteopenia, osteomalacia, Paget's disease, rickets, Cushing's syndrome, Fanconi's syndrome, Menkes' syndrome, Turner syndrome, Gaucher disease, Marfan's syndrome, osteogenesis imperfecta (OI), hyperparathyroidism, breast cancer metastatic to bone, multiple myeloma, hypoparathyroidism, hyperthyroidism, hypogonadism, thyrotoxicosis, scurvy, calcium deficiency, systemic mastocytosis, adult hypophosphatasia, hyperadrenocorticism, osteogenesis imperfecta, diabetes, rheumatoid arthritis, epilepsy, primary biliary cirrhosis, chronic obstructive pulmonary disease (COPD), hepatobiliary disease, distal renal tubular acidosis and chronic renal failure.

The term "cancer metastatic to bone" refers to the spread of cancer cells from their original location (breast, prostate) to bone. The tumor cells grow and multiply in the bone, eventually causing thinning of bone in the area where the cancer cells are deposited, bone pain and, in some cases, a fractured bone.

The term "fluid" as used herein is intended to describe a composition having sufficient viscosity so as not to disperse from the space it is intended to fill, yet having a viscosity low enough to be able to be administered via syringe.

The term "viscosity" refers to the property of resistance to flow in a fluid or semi-fluid. Viscosity is measured in a unit known as a poise. Suitable viscosities of the final solution mixture of the pharmaceutical composition for each particular application may readily be established by the skilled person, but will generally be in the range of about 10 to about 500 poises, preferably about 30 to about 200 poises.

This term "implantation" refers to the insertion of the composition of the invention into a mammal, preferably a human, whereby the implant serves to replace, fully or partially, tissue that has been damaged or removed.

Another aspect of implantation is also taken to mean the use of the composite to anchor or secure prostheses to bone, *in situ*. Am implant of the invention comprising a synthetic apatite and at least one anti-resorptive agent may serve to Furthermore the implant may be used as a vehicle to transport therapeutic agents to a certain site in a patient. In this aspect there is also included the adsorption onto the composite of a bioactive agent selected from growth factors, cytokines, chemotherapeutic drugs, enzymes, anti-microbials, anti-

inflammatory agents. An implanted material may be powder, fluid, semi-fluid or solid material.

The term "injection" refers to the insertion of a composition of the invention into a mammal using a syringe or other device which allows administration of the composition directly to the site of treatment. The composition serves to replace, fully or partially, tissue that has been damaged or removed. In particular, the composition of the invention is intended to fill a void in a bone, due to disease such as osteopenia or osteoporosis or to damage such as a fracture or non-union. Another aspect of injection is also taken to mean the use of the composite as a vehicle to transport therapeutic drugs and bioactive agents to a certain site in a patient. The bioactive agents are suitable for the treatment and relief of inflammation, infection, metastases or pain. Such factors may be too small to be effectively retained within the composition and hence may be introduced in the form of slow-release or controlled-release formulations into the composite to provide for their efficacy.

According to one currently preferred embodiment of the present invention the composite comprises a synthetic apatite and at least on anti-resorptive agent.

According to one currently more preferred embodiment the at least one anti-resorptive agent is a bisphosphonate or bisphosphonate derivative or salt or ester thereof.

According to one currently most preferred embodiment the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate or bisphosphonate derivative or salt or ester thereof.

In certain circumstances local administration of bisphosphonate is preferred over systemic delivery. Non-limiting examples include maintaining integrity of a prosthesis and treating or preventing periprosthetic bone resorption. Another exemplary indication is to prevent local bone loss due to absence of weight bearing resulting from injury or trauma. Systemic administration of bisphosphonates to patients having bone loss due to the absence of weight bearing resulting from a bone injury is well known in the art. Patients with renal disease, digestive disorders and other indications may not be candidates for systemic delivery of bisphosphonates and would benefit from local delivery.

A currently preferred embodiment of the present invention is an implant comprising a synthetic apatite and at least one anti-resorptive agent.

Additionally the composite may further comprise at least one bioactive agent, said bioactive agents including antibiotics and antiviral agents; chemotherapeutic agents; anti-rejection agents; analgesics and analgesic combinations; anti-inflammatory agents or hormones such as steroids.

Further provided by the present invention is a composition comprising the composite impregnated with a drug or agent able to deliver high tissue levels of said drug to the site of injured or diseased bone or to a tissue associated with bone. A composite of this type is particularly useful for, but not limited to, delivering antibiotic therapy to osteomyelitis patients.

The mineral component of bone is predominantly made of calcium phosphates. "Hydroxy apatite" refers to a highly crystalline calcium phosphate having the chemical formula: $Ca_5(PO_4)_3OH$. Hydroxy apatite and other highly crystalline synthetic apatite materials are considered to be less than optimal bone graft implants since, although they are biocompatible, they are for the most poorly biodegradable. The mineral fraction of natural bone is primarily composed of "poorly crystalline apatite", a calcium phosphate derivative. It is to be understood that that a part of the calcium (Ca^{++}) ions may be replaced with other divalent ions selected from the group consisting of Magnesium (Mg^{++}) and Zinc (Zn^{++}). The incorporation of additional or different divalent ions imparts on the composition certain properties which may be advantageous in certain applications.

In preferred embodiments the present invention provides a pharmaceutical composition comprising a synthetic apatite and at least one bisphosphonate composite further comprising a pharmaceutically acceptable carrier or excipient. According to one currently more preferred embodiment of the present invention a pharmaceutical composition comprising a synthetic apatite and anti-resorptive agent composite, at least one carrier having sufficient fluidity to enable injection of the composition to the site of treatment.

The pharmaceutical composition of the present invention is useful for treating bone diseases and disorders wherein there is need to fill a void in a bone or a need to anchor a prosthesis or implant to bone. In accordance with the invention use of the composite of the invention for the manufacture of a medicament is provided as a graft for diseased or traumatized bone useful in orthopedic, periodontal and craniofacial indications wherein the composite is provided alone or comprising bioactive agents..

In certain applications, a solid implant is desired. Further provided is a bone composition comprising a synthetic apatite, at least one amino acid in monomeric or polymeric form, further comprising at least one bisphosphonate, optionally further comprising a bioactive agent, further comprising an additive that promotes hardening of said composition *in situ* over about 24 hours following injection. Alternatively, in the reconstruction of structural tissues like cartilage and bone, certain applications may require implantation of a solid implant. This may be achieved by molding of the composition into three dimensional configuration articles of varying thickness and shape *in vitro*, prior to implantation. Accordingly, an implant is provided comprising a synthetic apatite, at least one amino acid in monomeric or polymeric form, further comprising at least one bisphosphonate, optionally further comprising a bioactive agent, further comprising an additive that promotes hardening of said composition which may be formed to assume a specific shape. The shapes include a sphere, cube, rod, tube or a sheet which may constitute a prosthesis. A non-limiting example of a hardener includes calcium sulfate or calcium phosphate compounds. The shape is determined by the shape of a mold or support which may be made of any inert material and may be in contact with the composite on all sides, as for a sphere or cube, or on a limited number of sides as for a sheet.

According to one aspect of the present invention the synthetic apatite of the composite is a poorly crystalline apatite. According to one exemplary embodiment the composite is prepared by microwaving calcium and phosphate ions with at least one anti-resorptive agent using a procedure disclosed in US Patent 6,231,607. The powder resulting from that process consists of poorly crystalline apatite (PCA) and anti-resorptive agent aggregates having a size of approximately 0.45 μm to 6μm in diameter, more preferably 1 μm-4 μm in diameter. The aggregates are comprised of crystals of approximately 0.20 μm-0.30 μm in size.

Another embodiment of the present invention provides a process for the preparation of a bone graft composite comprising the poorly crystalline apatite according to US Patent 6,231,067 comprising adding *ab initio* at least one additional anti-resorptive agent, and optionally adding at least one bioactive agent. The process for preparing the composition comprises sterilizing the powder, adding a sufficient amount of liquid to hydrate and disperse the powder, adsorbing a bioactive agent and preparing the wetted powder for administration. Following the wetting procedure the composition may be optionally

filtered to remove excess liquid, thus leaving a paste-like material on the filter.

The process comprises the following steps:

a) preparing a liquid mixture comprising ionic calcium, phosphate, at least one amino acid in either monomeric or polymeric form, a carbonate and at least one anti-resorptive agent;

b) subjecting said mixture to microwave irradiation;

c) quenching said irradiated mixture;

d) filtering said irradiated mixture so as to separate between the filtrate and a cake;

e) drying said cake;

f) grinding said dried cake into a powder;

g) sterilizing said powder;

h) optionally wetting said sterilized powder with a solution optionally comprising at least one bioactive agent;

i) preparing said wetted powder for administration.

According to one currently preferred embodiment of the present invention the powder resulting from step (f) consists of poorly crystalline apatite (PCA) calcium phosphate and anti-resorptive agent composite aggregates having a size of approximately 0.45 μm to 6μm in diameter, more preferably 1 μm-4 μm in diameter. The aggregates are comprised of crystals of approximately 0.20 μm-0.30 μm in size.

The mixture of step a) comprises a calcium ion that may be, for example, calcium chloride added to a concentration of about $5x10^{-3}$ to about $5x10^{-2}$. The preferred concentration is about $1x10^{-2}$. The phosphate may be a phosphate such as NaH_2PO, added to a concentration of about $3x10^{-3}$ to about $2x10^{-2}$. The preferred concentration is about $6x10^{-3}$. The amino acid may be any monomeric or polymeric amino acid but is preferably L-aspartic acid, added to a concentration of about 10-50 ppm. The preferred concentration is about 25 ppm. The carbonate may be, for example $NaHCO_3$, added to a concentration of about 1-600 ppm. The preferred concentration of carbonate is about 5-150 ppm. More than one carbonate compound may be used.

According to one currently preferred embodiment of the present invention the powder resulting from step (f) consists of poorly crystalline apatite (PCA) calcium phosphate aggregates having approximately 0.45 μm to 6 μm in diameter, more preferably 1 μm-4 μm in diameter.

The microwave step is typically carried out in a standard kitchen microwave (approximately 700W-1000W) for approximately 10-30 minutes.

The present invention provides a unique composite with superior properties for use as a bone graft in indications where a space filling material is needed.

The composition should be sterilized for use *in vivo*, in particular for use in clinical and therapeutic applications in mammals. Preferably the composite powder is sterilizable by ionization, preferably γ-irradiation, and retains its original X-ray diffraction pattern. The powder resulting from step (f) is irradiated at a minimum of 2.5 Mrad according to known GMP production procedures, followed by X-ray diffraction analysis.

A currently preferred embodiment of the present invention provides wetting the sterilized PCA powder with a pharmaceutically acceptable liquid such as water or a physiological fluid preferably comprising a bioactive agent. The liquid is added in a sufficient amount to allow wetting and dispersion of the powder to form a wetted mixture having the consistency of a paste or putty. In one currently preferred embodiment of the present invention the powder is mixed with liquid in a ratio of about 1:1 w/w or w/v to yield a paste-like substance. In one particular exemplary embodiment 0.3 gm powder is mixed with 0.3 ml sterile water comprising a therapeutically effective amount of antibiotic to yield approximately 0.5ml paste-like composition.

Alternatively, a sufficient amount of liquid is added to permit wetting and dispersion of the powder to form a hydrated precursor mixture having a consistency compatible with application to a filtration device. The wetted powder is filtered through a sterile filtration device having pore size enabling retention of the crystalline aggregates on the filter. Preferably, the pore size of the filtration device permits full retention of the bone graft material. In one particular exemplary embodiment 0.3 gm PCA powder are mixed with 2 ml sterile PBS. The slurry was filtered through a 0.45 μm filter to remove excess liquid to yield approximately 0.5 ml paste-like material.

The filtered material is left sufficiently wetted in order to enable handling without fragmentation or crumbling or separation of the liquid from the solid phase. Preferably the

wetted powder has a consistency or putty or paste. Preferably the paste has a viscosity in the range of 10-500 poises, more preferably 30-200 poises.

In another embodiment of the present invention, the wetted powder is blended under sterile conditions to a consistency compatible with administration to a lesion. The paste may be administered manually or with a spreading instrument such as a spatula. More preferably, the wetted powder is inserted into a syringe and is prepared for local administration or injection into the site of the defect or lesion.

According to the principles of the present invention the composite of the invention may be used in orthopedic indications including periodontal surgery, and plastic and craniofacial surgery. In a non-limiting example, the composite of the present invention may be used for augmentation of the alveolar ridge to facilitate retention of denture and to fill various periodontal lesions. It can also be used in to fill the gap in cases of bony defects, cysts and traumatic bone loss. The composite of the present invention may be used for maxillofacial dysplasia, filling of bone defects in skull, zygomatic and mandibular area and augmentation of various bony areas. In addition, the composite of the present invention may be used to reconstruct the calvaria including repair of cranial base and temporal bone defects following surgery. Orthopedic applications in which the compositions of the invention are useful include, but are not limited to, fractures and non-union fractures resulting from a trauma or generated by surgical means, percutaneous vertebral augmentation (PVA), hip resurfacing or spinal fusion in indications such as osteopenia or osteoporosis.

Anti-resorptive agents belonging to the group of bisphosphonates have been shown to be useful as anti-tumor drugs (Neville-Webbe et al., Cancer Treat Rev, 28(6):305-19, 2002) and may function as apoptosis inducers or cell growth and metastases inhibitors.

Pharmacology

The term "therapeutic" refers to any pharmaceutical, drug or prophylactic agent which may be used in the treatment (including the prevention, diagnosis, alleviation, or cure) of a malady, affliction, disease or injury in a patient.

The term "excipient" as used herein refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and

types of starch, cellulose derivatives, or gelatin. Pharmaceutical compositions may also include one or more additional active ingredients.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, grinding, pulverizing, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

The term "physiologically acceptable liquid carrier" or "diluent" refers to an aqueous or non-aqueous fluid that is well suited for injection preparations.

The composite of the experiment may be used in particle or powder form, or may be combined with a physiological liquid for use as a paste-like material. The composition may further comprise hardening agents for in situ or *in vivo* hardening that results in a molded body.

The pharmaceutical composition of this invention may be administered as a paste, preferably as an injectable paste, more preferably as an injectable paste that hardens *in situ,* within 24 hours following implantation. Alternatively an implant comprising the composite of the invention is provided. Furthermore, the composite may be used as a coating on synthetic or other implants such as pins and plates, for example, in hip replacement procedures. Thus, the present invention further provides implants or medical devices coated with the matrix of the invention.

According to an alternative embodiment the pharmaceutical composition further comprises at least one agent that renders the composition non-fluid under physiological conditions. A non-limiting example of a collagen that gels at physiological temperatures is disclosed in WO 00/47130.

Kits

The present invention further provides a kit comprising the disclosed bone graft composite, where the dry and liquid components may be present in separate containers in the kit, or some of the components may be combined into one container.

Further provided is a kit comprising the poorly crystalline apatite, where the dry and liquid components may be present in separate containers in the kit, or some of the components may be combined into one container.

Examples

Example 1: Preparation of Bone graft powder

A method for preparation of the bone graft is disclosed in US patent 6,231,607. The bone graft prepared by this method has an X-ray diffraction pattern similar to natural bone. The inventors now disclose that anti-resorptive agents such as bisphosphonates may be added during the formation of the poorly crystalline apatite crystals. The powder can be formulated into a fluid composition having advantageous properties for use as a bone graft material.

Materials and Methods

Graduated bottles, 2-liter capacity and 500 ml capacity

Glass ice bath

Microwave Oven (700W-1000W)

Vacuum Filter

Millipore Filter 0.45μm- 9 cm diameter Z29078-5 (Sigma)

Oven

Trizma buffer PRE-SET Crystals Type 7.4-FT (Sigma)

Sodium dihydrogen phosphate monohydrate $NaH_2PO_4 \cdot H_2O$ (MERCK)

Sodium Bicarbonate $NaHCO_3$ (ICN)

L-Aspartic Acid monosodium salt (Sigma)

Calcium Chloride Dihydrate $CaCl_2 \cdot 2H_2O$ (MERCK)

DDW/TDW, Filtered.

1) Two solutions were prepared:

Solution I ($CaCl_2$ + Trizma + bisphosphonate): 20.0 gr. Trizma, 2.94 gr. $CaCl_2$, bisphosphonate, and 2.0 liters of DDW.

Solution II (NaH_2PO_4 + Trizma): 20.0 gr. Trizma, 1.66 gr $NaH_2PO_4 \cdot H_2O$, 0.6 gr. $NaHCO_3$ 0.1 gr. L-Aspartic Acid, 2.0 liters of DDW.

2) Equal volumes of solution I and solution II (1.5 liters each) were mixed rapidly in a 4 liter glass bowl. The final solution had the following concentrations: 0.01M $CaCl_2$, 0.006M NaH_2PO_4, 150 ppm (mg/Liter) $NaHCO_3$, 25 ppm L-Aspartic Acid with varying concentration of bisphosphonate. A bisphosphonate, alendronate, was added as an acid or as a sodium salt.

3) The solution was heated in a microwave oven at maximum power for 30 min.

4) The irradiated mixture was placed in an ice bath for 1 hr.

5) The mixture was filtered through a Millipore filter (0.45μm).

6) The precipitate was washed with 50 ml of DDW.

7) The precipitate was transferred to a glass beaker and dried overnight at 55°-60°C.

8) The dried precipitate was ground to a fine powder using a mortar and pestle.

9) The resulting powder was weighed for quantity determination.

10) The powder was sterilized by γ irradiation.

11) The powder was stored in a closed sterile glass vial and labeled according to date, batch number and quantity.

A 3-liter batch generally yielded 1.5 gm composite. The bisphosphonate was added in the amounts of 0.0005mg 0.05 mg or 0.5 mg per liter. The amount of anti-resorptive agent can be adjusted for different applications and requirements. The skilled artisan will be able to determine the appropriate concentration of bisphosphonate in order to maximize the local effect while minimizing toxicity.

Example 2: X-Ray Diffraction analysis

Bone graft composite was prepared according to Example 1 and was exposed to X-ray analysis. An X-ray diffraction (XRD) pattern was obtained from a packed powder sample of the material pulverized in a mortar and pestle. X-rays were performed using an X-ray powder diffractometer, Rigaku, Japan. The scan rate was set to 0.5 degree/minute over the 2 theta (2θ) angular range from 20°-35°. The step size was set to 0.05°. Figure 1A shows the X-ray diffraction pattern of the bone graft material prepared without bisphosphonate. Note the large undifferentiated peak at 2θ=31°-33°. The X-ray diffraction pattern of a composite prepared with 0.0005 mg alendronate/liter is presented in figure 1B. The X-ray diffraction pattern of a composite prepared with 0.05 alendronate or 0.5 mg alendronate/liter are shown in Figures 1C and 1D respectively. In all the cases the X-ray diffraction patterns of the composites show strong similarity to the parent material, specifically a characteristic undifferentiated peak at 2 theta 2θ=31°-33°. The composite comprising the higher concentration of alendronate (0.5mg/l) exhibits a higher crystallinity pattern with an additional peak at 2 theta 2θ=24.24° and a larger peak around 2 theta 2θ=25.9°.

Example 3: Scanning Electron Microscope Analysis

Bone graft composites prepared with various amounts of different bisphosphonates are subject to Scanning Electron Microscope Analysis (SEM) in order to analyze aggregate structure.

Example 4: Level of Bisphosphonate in Composite

The level of bisphosphonates in certain indications a solid bone graft device is preferred. present in the composite of the invention is determined by methods known in the art including capillary electrophoresis and liquid chromatography techniques.

Example 5: Resorbability Assay

Resorbability of the composite is attributable in part to its crystallinity and chemical composition. Several assays, both *in vivo* and *in vitro* are known in the art to analyze resorbability of implantable composites. In vivo assays include intramuscular, subcutaneous and intraosseous models. An in vivo assay measuring subcutaneous resorption of dense carbonate apatite is disclosed in Barralet (Barralet, J. et al., J Biomed Mater Res 49(2):176-82, 2000). Another example discloses the resorption of porous ceramic implants in a dog model (Pollick, S., et al., J Oral Maxillofac Surg, 53(8):915-22; 1995).

Example 6: Injectable Composition

These examples demonstrate the methods of preparing a fluid composition for use as a bone graft material.

An amount of 0.3 gm dry sterile powder of Step f) in example 1 was mixed with 2ml sterile PBS. The composition was mixed for 1 hour on a shaker and filtered through a 0.45 μm membrane to remove excess liquid. Remaining on the filter was approximately 0.5 ml of a pasty substance that was placed into a syringe for local administration in an animal model.

An amount of 3 gm dry sterile powder of Step 1 was mixed with 3 ml sterile PBS to yield approximately 5 ml of a pasty substance that was placed into a syringe for local administration in an animal model.

Additional compositions are prepared by varying the w/w or w/v ratio of the composite and a pharmaceutically acceptable diluent. Viscosity of the fluid or semi-fluid compositions was determined by standard techniques.

A composition comprising the composite of the invention may be cast into a multiplicity of different shapes and forms for use as an implant. Certain hardeners including natural and synthetic polymers or calcium sulfate may be added to induce hardening of the composition.

Example 7: Biomechanical Testing

The powder, cement and implants of the present invention are tested in several systems to determine biomechanical properties including:

Viscosity: the resistance of a substance to flow under stress;

Ultimate Tensile Strength: the maximum stress that a material can withstand before failure in tension;

Bond Strength Between Prosthesis and Bone: the load required to fracture the bond divided by the cross-sectional area of the bond. Bond strengths are measured either in shear or tension.

Example 8: *in vitro* and *in vivo* Assays

The materials of the present invention are tested in several models including cell assays and animal models. Non-limiting examples include *in vitro* osteoclast resorption (i.e. Taylor et al., Int J Oral Maxillofac Implants, 17:321-30, 2002), maintenance of alveolar bone following tooth extraction (Denissen, et al, J Periodontol, 71:279-86, 2000), repair of a segmental defect in a canine femur (Fujibayashi et al., J Long Term Eff Med Implants;11:93-103, 2001), inhibition of debris induced osteolysis caused by prosthesis loosening (Schwarz et al., J Orthop Res,18(6):849-55, 2000).

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, including the contents of the references cited herein, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

Claims

1. A bone graft composite comprising a synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate and at least one anti-resorptive agent, wherein the at least one anti-resorptive agent is introduced into the composite *ab initio*.

2. The bone graft composite according to claim 1 wherein said at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

3. The bone graft composite according to claim 2 wherein the bisphosphonate is alendronate.

4. The bone graft composite according to claim 1 wherein the synthetic apatite is a poorly crystalline apatite.

5. The bone graft composite according to claim 1 wherein the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate.

6. The bone graft composite according to claim 1 further comprising at least one bioactive agent.

7. The bone graft composite according to claim 6 wherein said at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

8. The bone graft composite according to claim 1 having an X-ray diffraction pattern comprising a broad peak at 2 theta values of about 31°-33°.

9. A pharmaceutical composition comprising a composite comprising a synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate, at least one anti-resorptive agent, wherein the at least one anti-resorptive agent is introduced into the composite *ab initio*, further comprising a pharmaceutically acceptable carrier or diluent.

10. The pharmaceutical composition according to claim 9 wherein said at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

11. The pharmaceutical composition according to claim 10 wherein the bisphosphonate is alendronate.

12. The pharmaceutical composition according to claim 9 wherein the synthetic apatite is a poorly crystalline apatite.

13. The pharmaceutical composition according to claim 12 wherein the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate.

14. The pharmaceutical composition according to claim 9 further comprising at least one bioactive agent.

15. The pharmaceutical composition according to claim 14 wherein the at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

16. The pharmaceutical composition according to claim 12 having an X-ray diffraction pattern comprising a broad peak at 2 theta values of about 31°-33°.

17. The pharmaceutical composition according to claim 16 further comprising at least one hardener.

18. The pharmaceutical composition according to claim 17 wherein the composition is fluid at ambient temperature and solid at physiological temperatures.

19. A method for treating orthopedic, periodontal and craniofacial indications comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising a composite comprising synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate, and at least one anti-resorptive agent, wherein the at least one anti-resorptive agent is introduced into the composite *ab initio*.

20. The method according to claim 19 wherein said at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

21. The method according to claim 20 wherein the bisphosphonate is alendronate.

22. The method according to claim 19 wherein the synthetic apatite is a poorly crystalline apatite.

23. The method to claim 19 wherein the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate.

24. The method according to claim 19 further comprising at least one bioactive agent.

25. The method according to claim 24 wherein the at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

26. The method according to claim 19 wherein the composition has an X-ray diffraction pattern comprising a broad peak at 2 theta values of about 31°-33°.

27. The method according to claim 19 further comprising at least one hardener.

28. The method according to claim 27 wherein the composition is fluid at ambient temperature and solid at physiological temperatures.

29. A method for anchoring a prosthetic device to a bone comprising administering to the prosthetic device an amount of a composition comprising a composite comprising synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate, at least one anti-resorptive agent, wherein the at least one anti-resorptive agent is introduced into the composite *ab initio*, further comprising at least one hardener to bind the prosthetic device to bone.

30. The method according to claim 29 wherein said at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

31. The method according to claim 30 wherein the bisphosphonate is alendronate.

32. The method according to claim 29 wherein the synthetic apatite is a poorly crystalline apatite.

33. The method to claim 29 wherein the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate.

34. The method according to claim 29 further comprising at least one bioactive agent.

35. The method according to claim 34 wherein the at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

36. The method according to claim 29 wherein the composition has an X-ray diffraction pattern comprising a broad peak at 2 theta values of about 31°-33°.

37. The method according to claim 29 wherein the composition is fluid at ambient temperature and solid at physiological temperatures.

38. Use of a composite comprising synthetic apatite, at least one amino acid in monomeric or polymeric form, a carbonate, at least one anti-resorptive agent, wherein the at least one anti-resorptive agent is introduced into the composite *ab initio*, for the manufacture of a medicament for treating diseased bone in orthopedic, periodontal and craniofacial indications.

39. Use according to claim 38 wherein said at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

40. Use according to claim 39 wherein the bisphosphonate is alendronate.

41. Use according to claim 38 wherein the synthetic apatite is a poorly crystalline apatite.

42. Use according to claim 38 wherein the synthetic apatite is a poorly crystalline apatite and the at least one anti-resorptive agent is a bisphosphonate.

43. Use according to claim 38 further comprising at least one bioactive agent.

44. Use according to claim 43 wherein the at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

45. Use according to claim 44 wherein the composition has an X-ray diffraction pattern comprising a broad peak at 2 theta values of about 31°-33°.

46. A method of preparing a bone graft composite comprising the steps of:

a) preparing a liquid mixture of ionic calcium, phosphate, an amino acid in either monomeric or polymeric form, a carbonate and at least one anti-resorptive agent;

b) subjecting said mixture to microwave irradiation;

c) quenching said irradiated mixture;

d) filtering said quenched mixture so as to separate between the filtrate and a cake;

e) drying said cake;

f) grinding said cake into a powder;

g) sterilizing said powder;

h) optionally wetting said sterilized powder with a solution optionally comprising at least one bioactive agent;

i) preparing said wetted powder for administration.

47. The method according to claim 46 wherein said liquid mixture of step a) comprises calcium chloride, sodium phosphate, L-aspartic acid, sodium carbonate and at least one anti-resorptive agent.

48. The method according to claim 47 wherein the at least one anti-resorptive agent is a bisphosphonate or pharmaceutically acceptable salt or ester thereof.

49. The method according to claim 48 wherein the bisphosphonate is alendronate.

50. The method according to claim 46 wherein the at least one bioactive agent is selected from the group consisting of antibiotics, antiviral agents, chemotherapeutic agents, anti-rejection agents, analgesics and analgesic combinations, anti-inflammatory agents and hormones.

For the applicant:

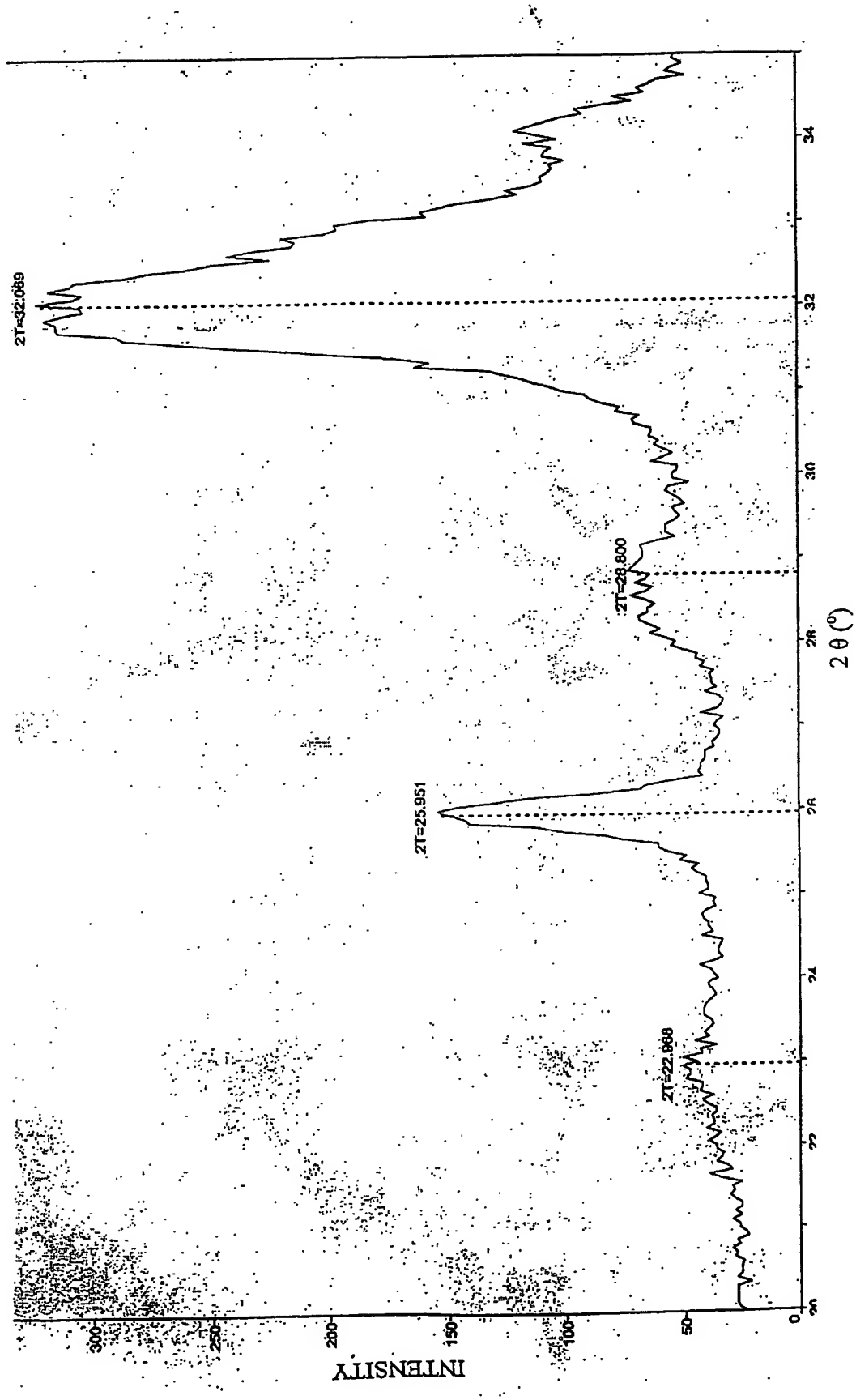
Cynthia Webb

Cynthia Webb, Ph.D.

Abstract

A bone graft composite material comprising a synthetic apatite and at least one anti-resorptive agent useful as a bone graft implant, methods of preparing said composite wherein said at least one anti-resorptive agent is introduced *ab initio*, and uses thereof are provided. The physical and biological properties of the composite are controlled by microwave irradiation to precipitate the composite and by the addition of certain anti-resorptive agents, as well as optional bioactive agents or hardeners. The composite may be used as a powder, a paste or an implant.

Figure 1A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2θ(°)
34

Figure 1B

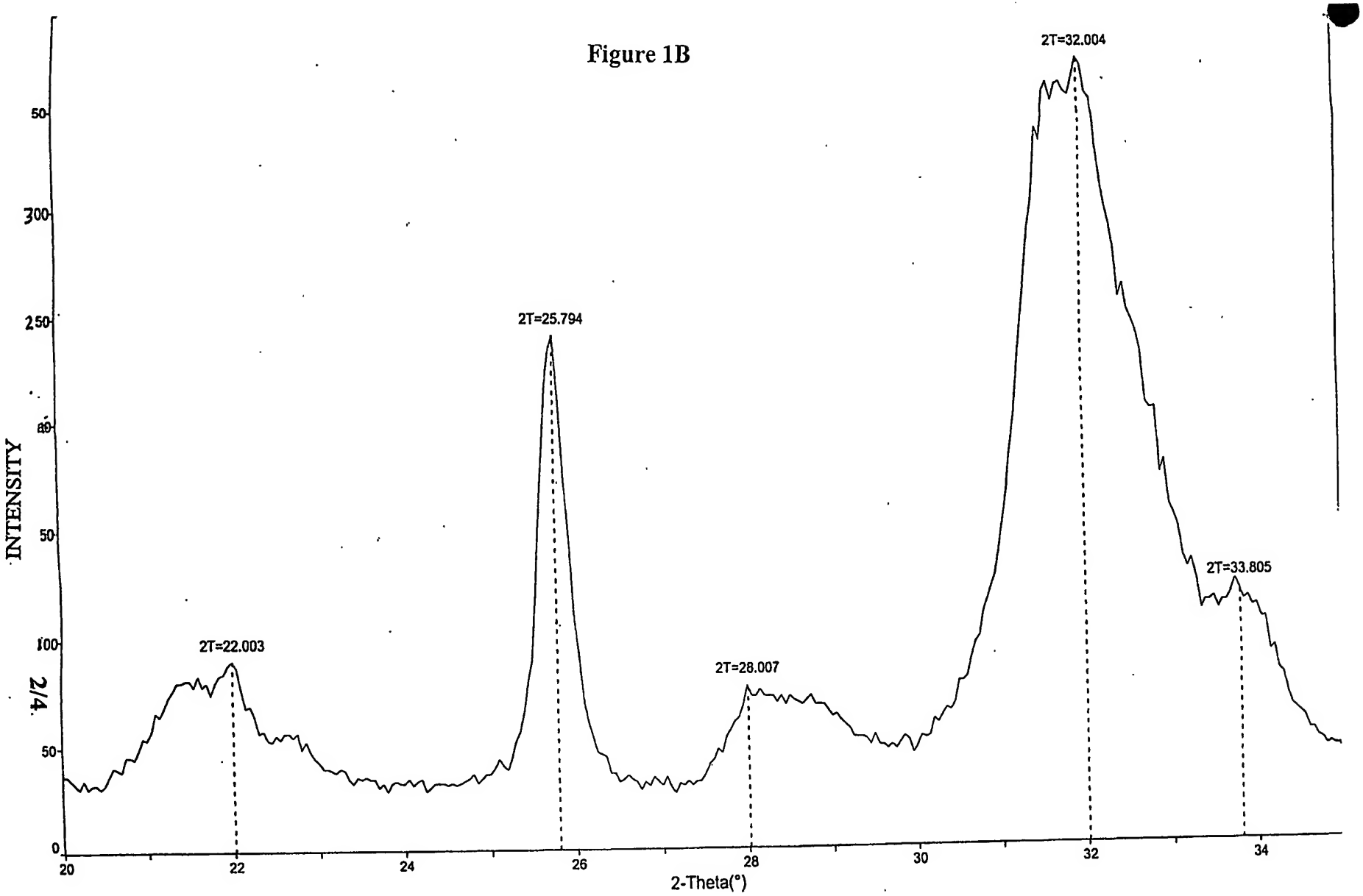
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Figure 1C

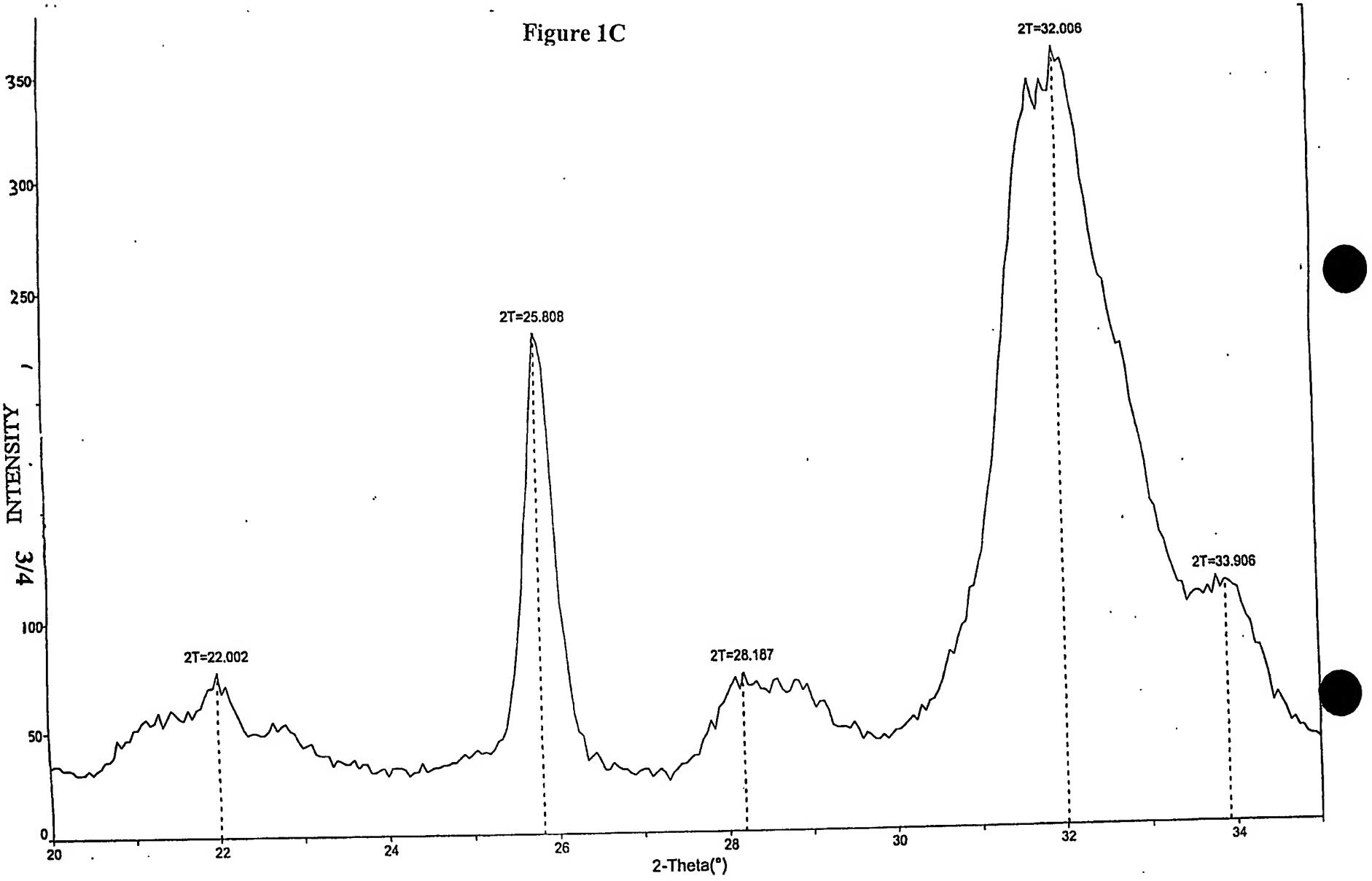
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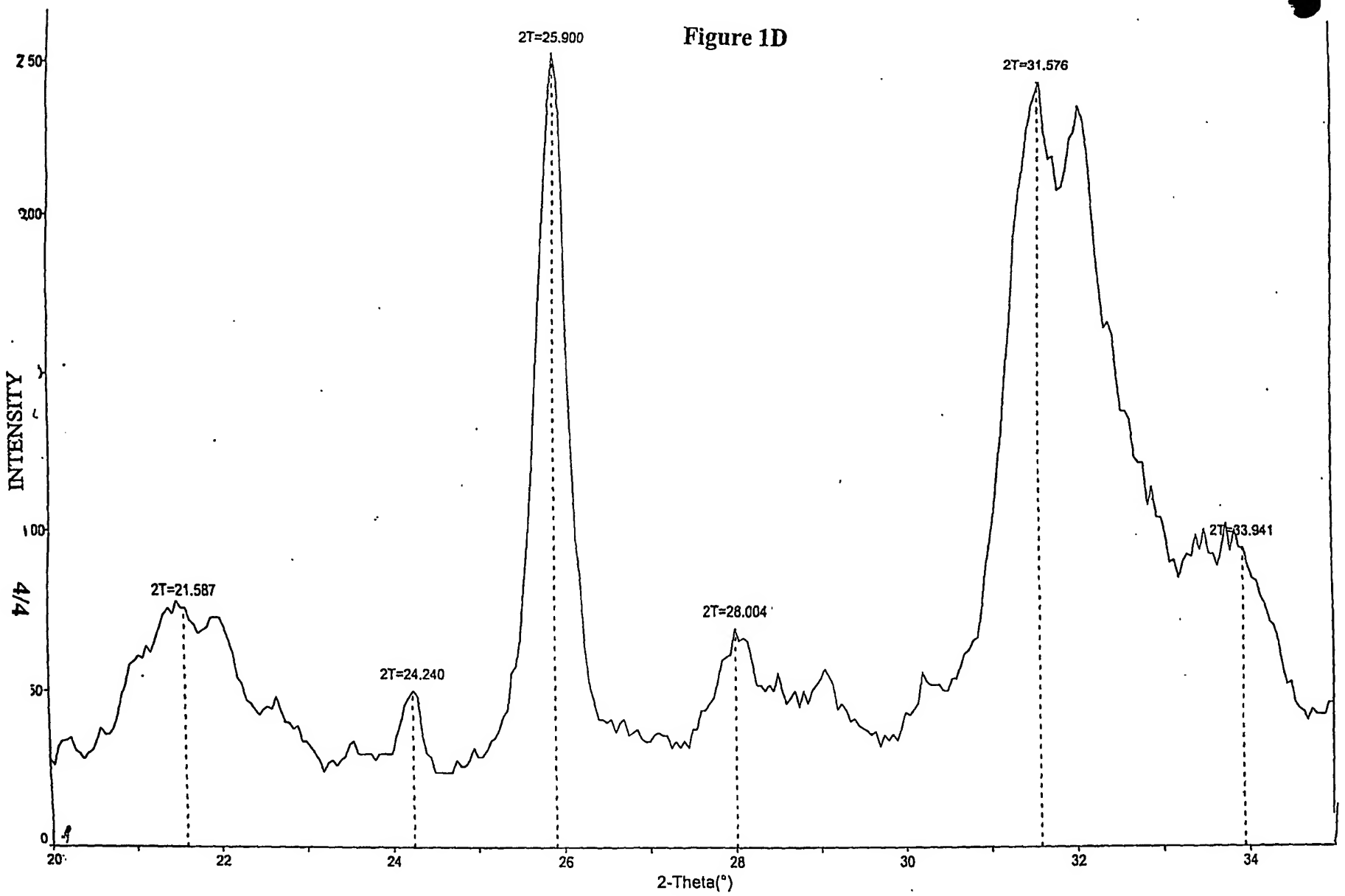
Figure 1D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